

("Clean paragraphs"):

Figures 5A and 5B show a comparison of the inventive assay to a conventional COBRA assay. Figure 5A (Panel A) shows a COBRA gel used to determine the level of DNA methylation at the *ESR1* locus in DNAs of known methylation status (sperm, unmethylated) and HCT116 (methylated). The relative amounts of the cleaved products are indicated below the gel. A 56-bp fragment represents DNA molecules in which the *TaqI* site proximal to the hybridization probe is methylated in the original genomic DNA. The 86-bp fragment represents DNA molecules in which the proximal *TaqI* site is unmethylated and the distal site is methylated. Figure 5B (Panel B) summarizes the COBRA results and compares them to results obtained with the methylated and unmethylated version of the inventive assay process. The results are expressed as ratios between the methylation-specific reactions and a control reaction. For the bisulfite-treated samples, the control reaction was a *MYOD1* assay as described in Example 1. For the untreated samples, the *ACTB* primers described for the RT-PCR reactions were used as a control to verify the input of unconverted DNA samples. (The *ACTB* primers do not span an intron). "No PCR" indicates that no PCR product was obtained on unconverted genomic DNA with COBRA primers designed to amplify bisulfite-converted DNA sequences.

Figures 6A-6C illustrate a determination of the specificity of the oligonucleotides. Eight different combinations of forward primer, probe and reverse primer were tested on DNA samples with known methylation or lack of methylation at the *ESR1* locus. Figure 6A (Panel A) shows the nomenclature used for the combinations of the *ESR1* oligos. "U" refers to the oligo sequence that anneals with bisulfite-converted unmethylated DNA, while "M" refers to the methylated version. Position 1 indicates the forward PCR primer, position 2 the probe, and position 3 the reverse primer. The combinations used for the eight reactions are shown below each pair of bars, representing duplicate experiments. The results are expressed as ratios between the *ESR1* values and the *MYOD1* control values. Figure 6B (Panel B) represents an analysis of human sperm DNA. Figure 6C (Panel C) represents an analysis of DNA obtained from the human colorectal cancer cell line HCT116.

IN THE CLAIMS:

Please cancel original claims 1-26, and, pursuant to 37 C.F.R. § 121(c), submit therefore new claims 27-64 as follows (applicants, to expedite the present examination, have attached hereto Appendix A that contains "marked-up" versions of the allowed claims as of prior "Amendment B" in the parent prosecution history, which have been amended to provide for subject matter supported in the specification and providing the full scope of the invention as conceived by the inventors):